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## Effect of Chitin and Chitosan in Improvement of Plant Growth and Anti-Fungal Activity

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THE STUDY aimed to improve the seed germination of cowpea, basil and cucumber by employing chitin and chitosan and to evaluate their effect on some plant pathogenic fungi. The results showed encouragement effect of chitin and chitosan on the seed germination of the three crops. Cowpea has more response to the chitin or chitosan with a significant difference in comparison to the control samples. Their germination percentages were 80% and 87% when treated with a concentration of 1mg chitin or chitosan, respectively. The germination percentages of basil seeds were 91% and 94% when employing 1mg/mL chitin or chitosan, respectively, in comparison to the control sample 76%. On the other hand, the generation percentage for cucumber seeds was 96% for both 1mg/mL chitin and chitosan as comparison to 76% for control sample. The germination percentage was decrease with increase of concentration of chitin or chitosan. The highest percentage of inhibition of pathogenic fungi was 70.58% for *Macrophomina* sp. for both 4 mg/ml chitin and chitosan. The lowest percentage of inhibition at a concentration of 1mg chitin was 8.23% for *A.niger*. While in the presence of 1mg/mL chitosan the lowest inhibition percentage was 11.76% for *Rhizoctonia solani*. It can be concluded that the percentage of germination for each of cowpea, basil and cucumbers decreases with increasing the concentration of chitin or chitosan. The percentage of inhibition of pathogenic fungi was increase with increase the concentration of chitin or chitosan.

**Keywords**: Anti-fungal activity, Bio-degradation polymers, Chitin, Chitosan, Plant pathogens, Seed germination.

### Introduction

In the recent decades, the control of plant diseases has become more difficult despite of several benefits of using of fungicides in the development of agriculture (Kim & Hwang, 2007). However, the increased use of these pesticides is a threat to the environment and human health, as well as resistance to fungal strains of pesticides, became a major problem (Gill &Garg, 2014).

There is a global trend to discover new alternatives processes to post-harvest plant control that prioritize methods that reduce disease incidence and prevent adverse and side effects on human health as a result of the excessive application of industrial fungicides (Bautista et al., 2006).

Biodegradation of natural compounds from animals and fungi are important for inhibition the activity of plant pathogens such as chitin or chitosan, which are high molecular weight polymers. Amino sugar is the basic unit in composition of these compounds, which gives the mechanical strength of the organism. The chitin can be found in cell wall of fungi and the skeletons of insects and crustaceans (Dutta et al., 2004).

Chitin and its derivative chitosan are non-toxic substances, vital active agents that can be used as antifungals in addition to inducing the defense

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mechanism in plant tissues. It can therefore be advocated that chitin and its derivative have a great potential for application in agriculture then it can be predicted that in the future these biopolymers will be used significantly to replace chemical pesticides or as growth regulators (Gooday, 1990). Several studies have demonstrated different applications for chitin and chitosan in plants and crops production. Among these applications are application in crops improving of production, visual and nutritional aspects, alleviate biotic and abiotic stresses (Mujtaba et al., 2020). Researchers also found, when employing chitosan, increase product shelf life (Pereira et al., 2017). Although these polymers show the potential to be applied in many crops, a few carriers have been explored as a matrix for plant growth promoters (Liu et al., 2013).

This study aimed to improve the seed germination of cowpea, basil and cucumber by employing chitin and chitosan and to evaluate their effect on some plant pathogenic fungi.

## Materials and Methods

*Material*

The materials were potato dextrose agar (LAB (England) and the seeds of plants used in the experiment were obtained from the laboratories of the Najaf Agriculture Directorate in Najaf, Iraq. The pathogenic fungal isolates were obtained and diagnosed in the Faculty of Agriculture, University of Kufa, Najaf, Iraq.

*Methods*

The chitin was prepared from Shrimp shells after several processes of cleaning, washing and drying of shells at room temperature. The flack shells were milled and powdered and the chitin was extracted by treating the powder with a solution of (1N) HCl by 30min. at 1:15 (W/V), the product was collected on filter paper and washed with water many times until the medium reaches pH 7. After that the powder was treated with 3% NaOH at 1:10 (W/V) and put it in the autoclave for 10min at 121°C and pressure 15Ibs. The collected powder on filter paper was washed with water many times until the medium reaches pH 7. The powder was treated with a solution of 32% sodium hypochlorite by 3min. and then the chitin is collected and washed with distilled water and dried in the oven for 4 hrs. at 60°C.

Chitosan was prepared from chitin powder by treating the chitin powder with 50% NaOH at 1:15 (W/V) and placed in the autoclave for 30min. at 121°C and pressure of 15lbs. The resulting chitosan was collected by filter paper and washed and dried at 60°C for 4hrs. in the oven.

*Effect of chitosan solutions on seed germination*

Seeds of basil, cowpea, and cucumber were sterilized by submerging them with 1% sodium hypochlorite and washed them with sterile distilled water. A number of 50 sterilized seeds of each plant were separately counted on filter paper in a laboratory plate of 8.5cm diameter. This method was replicated three times per treatment and 5mL distilled was adding water to the control plate. A five ml of each concentration of chitin and chitosan of 1, 2, 3 and 4mg/mL was added for each plate. All plates were placed in an incubator under conditions of growth under dark and temperature of 22°C. The number of seeds growing every 2 days was recorded from the second day and put the seeds on the filter paper in the Petri dish. The germination percentage is calculated from Equation 1:

$$\text{Percentage of germination} = \frac{\text{No.of grown seeds}}{\text{Total No.of seeds}} \times 100 \quad ..1$$

*Effect of chitin and chitosan against pathogenic fungi of the plant*

Several concentrations of chitin and chitosan of 1, 2, 3 and 4mg/mL were added separately to the pre-prepared potato dextrose agar (PDA) medium and cooled to 45°C under sterile conditions and thoroughly mixed and shaken and poured into a sterile Petri dish with a diameter of 8.5cm. Each dish inoculated with a piece of pathogenic fungal colony (0.5cm) for 7 days at a temperature of 25°C. The control treatment (PDA-free medium extracted) reaches to the maximum extent of dish fungal growth diameters were measured to find the percentage of inhibition by employing Equation 2:

$$\text{Percentage of inhibition} = \frac{\text{Growth rate in control} - \text{fungal growth rate}}{\text{Growth rate in the control}} \times 100 \quad ..2$$

*Statistical analysis*

The results of the experiments were analyzed using the complete randomized blocks design (C.R.B.D) and the averages were compared with the least significant difference (L.S.D) test at a probability level of 0.05.

## Results and Discussion

*Effect of chitin and chitosan on seed germination*

There was a significant effect of chitin and chitosan on the germination percentage of three randomly selected agricultural crops at probability 95% confidence interval. The results demonstrate the highly L.S.D.$_{0.05}$ was 23.59 for cowpea at 1mg/mL concentration of chitin with germination rate 80% as comparison to the control sample which was of 15% as shown in Table 1. The results in Table 1 showed germination percentages of basil and cucumber were 91% and 96%, respectively, when using chitin at a concentration of 1mg/mL. In addition, the result in the Table 1 showed that the rate of germination for all the crops was decreased with the increase of chitin concentration. At the concentration 4mg/mL of chitin, the germination rates were 26, 76 and 86% for cowpea, basil and cucumber, respectively. The images in Fig. 1 demonstrates clearly the difference in germination rate of cowpea (A), basil (B) and cucumber (C) of 1mg/mL chitin (on left) and control (on right), respectively.

**TABLE 1. Germination percentages of crops with different concentrations of chitin**

| Chitin concentration | Cucumber | Basil | Cowpea |
|---|---|---|---|
| 0mg/mL | 76 | 76 | 15 |
| 1mg/mL | 96 | 91 | 80 |
| 2mg/mL | 91 | 83 | 68 |
| 3mg/mL | 87 | 80 | 50 |
| 4mg/mL | 86 | 76 | 26 |
| L.S.D.( 0.05) | 9.48 | 9.60 | 23.59 |

The basil records have a good L.S.D.$_{0.05}$ of 9.6 with 1 mg/ml chitin at germination rate of 91% as comparison to 76% of control sample. While the cucumber seeds gave a germination rate of 96% at 1mg/mL chitin as comparison to 76% control sample with L.S.D.$_{0.05}$ of 9.48 as shown in Table 1.

The lowest germination rate was at a concentration of 4mg/mL chitin in comparison to the concentration of 2mg/mL chitin where the germination rates were 91, 83 and 68% for cucumber, basil, and cowpea, respectively, while at 3mg/mL chitin the germination rates were 87, 80 and 50% for cucumber, basil, and cowpea, respectively.

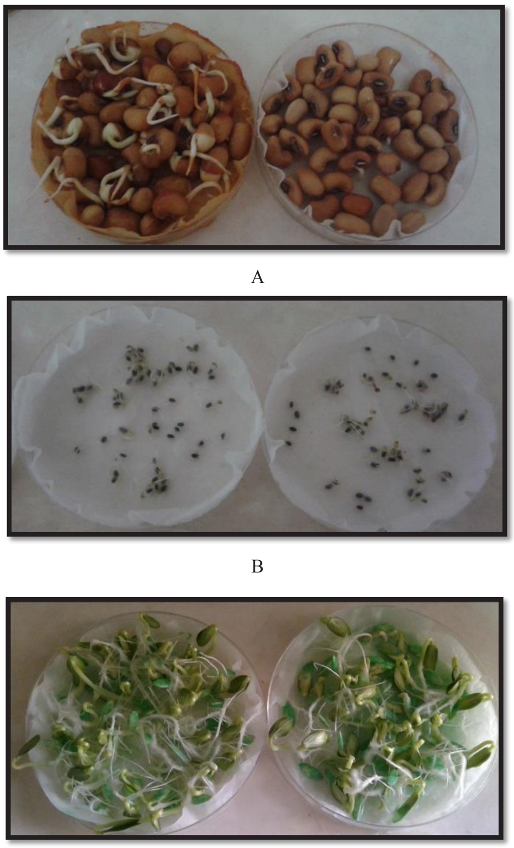
A
B
C

**Fig. 1. Germination rate of cowpea (A), basil (B) and cucumber (C) of 1mg/mL chitin (on left) and control (on right), respectively**

These results are agreed with what Liopa and his collogues have found that increasing of germination rate for parsley seeds at 1% chitin with variation in the germination rate at high concentration of chitin (Liopa-Tsakalidi et al., 2010).

The (L.S.D.$_{0.05}$) of 12.7 within cucumber germination rates at concentration of 1mg/mL chitosan which gave the highest germination rate 96% as comparison to 76% control sample. While the germination rates of cucumber were 91, 91 and 85% when the seeds were treated with 2, 3 and 4mg/mL of chitosan, respectively, as shown in Table 2.

The results demonstrated the highest germination rate of basil was 94% at 1mg/mL chitosan with L.S.D.$_{0.05}$ of 11 as comparison to 76% of the control sample. The germination rate of all crops in the presence of chitosan was decreased with increasing the concentration of chitosan.

**TABLE 2. Germination percentages of crops with different concentrations of chitosan**

| Chitosan concentration | Cucumber | Basil | Cowpea |
|---|---|---|---|
| 0mg/mL | 76 | 76 | 15 |
| 1mg/mL | 96 | 94 | 87 |
| 2mg/mL | 91 | 82 | 69 |
| 3mg/mL | 91 | 80 | 61 |
| 4 mg/mL | 85 | 79 | 48 |
| L.S.D.( 0.05) | 12.70 | 11.00 | 22.55 |

There was a variation in the optimum concentration of chitosan in improving of the germination percentage of cowpea, basil and cucumber crops as shown in Table 2. The result showed cowpea had more response to chitosan than basil and cucumber with germination rate of 87% at concentration 1mg/ml chitosan that has appeared $L.S.D._{0.05}$ of 22.55. The germination rates of cowpea were 69, 61 and 48% when treated with chitosan 2mg, 3mg and 4mg/mL, respectively.

The results showed that chitin and its derivatives chitosan had a positive effect in increasing the growth of plants at low economic cost. This result agrees with Burrows et al. who had found that treatment of soybean seeds before implanting with chitosan extract by using 0.5% HCl gave a good growth to the plant and resistance to fungal diseases (Burrows et al., 2007). Increasing the concentration of chitosan from 5 to 25ppm which was added to the nutrient solution of the orchid plant was directly proportional to increase of vegetative growth (Stevens, 2005) and improve of production quality (Uthairatanakij et al., 2007). The results of Zeng and Shi showed that the coverage of rice seeds with chitosan had a significant effect in increasing the germination rate by 5% (Zeng & Shi, 2009). The use of chitosan at a concentration of 500mg/L in potato nutrient solutions led to a significant increase in number and yield of potato production as comparison to the control (Asghari-Zakaria et al., 2009). Lizárraga-Paulín et al. (2011) explained that corn seed coverage with chitosan gave a positive result in stimulating germination. All these previous studies are supporting our results in this study. Rahman and his colleagues approved that root length and plant height were increased significantly when employed chitosan on canopy of field grown strawberry plants (Rahman et al., 2018) and the total carotenoids contents in strawberry fruit increased with spray application of chitosan in a dose-dependent manner up to 1000ppm.

*Effect of chitin and chitosan against some plant pathogenic fungi*

There was a significant difference of chitin and chitosan at probability 95% confidence interval on the inhibition rates of some plant pathogenic fungi. The inhibition results of the plant pathogenic fungi showed that the inhibition rate increased by increasing the concentration of chitin and chitosan as shown in Table 3. The highest inhibition rate was 70.58% for *Macrophomina* sp. with a good difference at $L.S.D._{0.05}$ of 12.42 for 4mg/mL chitin or chitosan concentration. The inhibition rates of chitin were 20, 35.29 and 48.23% for the concentration of 1, 2 and 3mg/mL chitin, respectively. *Aspergillus niger* is the most resistant fungus to the concentration of 4mg/mL chitin and chitosan with inhibition rate 17.64% and 29.41% for chitin and chitosan, respectively, as shown in table 3 with $L.S.D._{0.05}$ of 4.07. The inhibition rates ranged from 8.23 to 14.11% for the concentrations of 1 and 3mg/mL chitin, respectively, while the ratios ranged from 15.29% to 23.52% for the concentration of 1 and 3mg/mL chitosan.

The results shown in Table 3 that *A. flavus* gave 41.17% of inhibition with 4mg/mL chitin concentration and 35.29% with 4mg/mL chitosan concentration at $L.S.D._{0.05}$ of 4.82. The inhibition rates ranged from 17.64% to 35.29% for the concentration of 1 and 3mg/mL chitin and these ratios were comparable with the inhibition rates of 1 and 3mg/ml chitosan where it was estimated between 18.82%- 31.76%.

The ($L.S.D._{0.05}$) of 5.85 within the inhibition rate of *Rhizoctonia solani* was 41.17% at 4mg chitin and 29.41% at 4mg chitosan. The inhibition rate was 23.52, 32.94, 41.17% at 1, 2, and 3mg chitin, respectively, while the inhibition rate of chitosan was 11.76, 23.52, 25.88% at 1, 2, and 3mg chitosan, respectively.

The fungus *F.oxysporum* gave the highest inhibition rate 40% for both 4mg chitin and 4mg chitosan with $L.S.D._{0.05}$ of 5.2, while the lowest inhibition rate was 20% at concentration 1mg chitin and 1mg chitosan.

**TABLE 3. Percentages of inhibition of fungal growth obtained using different concentrations of chitin and chitosan**

| Concentrations | *F.oxysporum* | *Rhizoctonia solani* | *Macrophomina* spp. | *A.flavus* | *A.niger* |
|---|---|---|---|---|---|
| 1mg/mL chitin | 20.00 | 23.52 | 20.00 | 17.64 | 8.23 |
| 1mg/mL chitosan | 20.00 | 11.76 | 17.64 | 18.82 | 15.29 |
| 2mg/mL chitin | 33.33 | 32.94 | 35.29 | 29.41 | 11.76 |
| 2mg/mL chitosan | 26.66 | 23.52 | 29.41 | 29.41 | 18.82 |
| 3mg/mL chitin | 37.33 | 41.17 | 48.23 | 35.29 | 14.11 |
| 3mg/mL chitosan | 40.00 | 25.88 | 47.00 | 31.76 | 23.52 |
| 4mg/mL chitin | 40.00 | 41.17 | 70.58 | 41.17 | 17.64 |
| 4mg/mL chitosan | 40.00 | 29.41 | 70.58 | 35.29 | 29.41 |
| L.S.D. (0.05) | 5.2 | 5.85 | 12.42 | 4.82 | 4.07 |

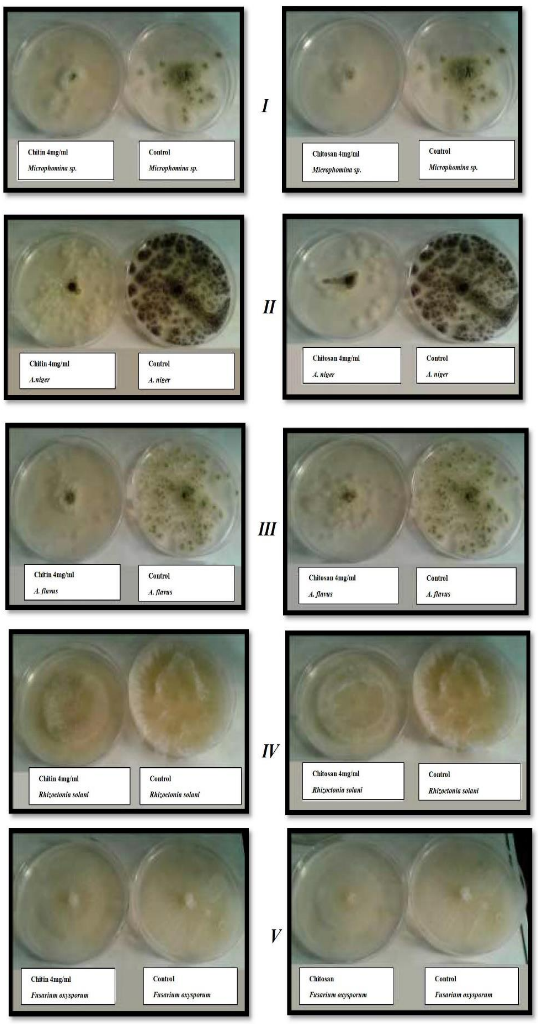


**Fig. 2. Antifungal effect of chitin and chitosan against pathogenic fungi of the plant [(I) *Macrophomina* sp., (II) *A. niger*, (III) *A. flavus*, (VI) *Rhizoctonia solani* and (V) *F. oxysporum*, respectively]**

In general, chitin and chitosan have shown varying efficacy in inhibition of pathogenic fungi and the highest pathogenic inhibition percentages for all fungi were obviously appeared with 4mg/mL concentration of chitin or chitosan as shown in the Fig. 2.

This effect related to chitin and its derivatives that work indirectly by making the $Ca^{2+}$ ion unavailable, which is a necessary nutrient for the growth of fungi. These compounds also work as chelating agents which interfere with plasma membrane compounds leading to alteration in the permeability of the fungal membrane, a pronounced inhibition of low molecular weight chitosan was observed for a number of fungi (Hirano & Nagao, 1989). The treatment of tomato seeds with chitosan stimulated the plant's defensive mechanism against the infection of *F.oxysporum* because the chitin and its derivatives has free amino acids that stimulate the antifungal activity (Benhamou et al., 1994). Chitin derivatives are particularly highly functional groups such as chitosan and derivative compounds that have an antifungal activity that works directly on plant pathogens. This effect depends on the chemical properties of the compound and concentration, in other words when using a 1g/L chitosan polymer it completely inhibits the growth of fungal mycelium for *Rhizotonnia solan*. Such inhibition is reduced to 80% when chitosan at a concentration of 500mg/L is used as well as used as a fungicide against bean seed infection and a plant growth stimulator (Burrows et al., 2007). Chitosan was studied antifungal effect on red grapes and honey melons to inhibit growth of *Penicillium chrysogenum, Fusarium oxysporum, Aspergillus parasiticus, Aspergillus fumigatus and Aspergillus niger* and was found that chitosan is suitable to use as an antifungal edible film in the food industry in the near future (Irkin & Gludas, 2014). Lee and his colleagues demonstrated that the antifungal activity of chitosan was dependent both the its MW and concentration. They found that the highest antifungal activity against *P.italicum*. Furthermore, the antifungal activity was significantly reduced in the presence of $Ca^{2+}$, whereas its effect was recovered by ethylenediaminetetraacetic acid, suggesting that the medium of chitosan acts via disruption of $Ca^{2+}$ gradient required for survival of the fungus (Lee et al., 2016).

## Conclusion

Chitin and chitosan have a significant effect on the germination rate of cowpea, basil and cucumber which was increased with the decrease of the concentration of chitin and chitosan. Cowpea has the better response of germination rate than cucumber and basil. Inhibition of pathogenic activity of some plant fungi was related to the owing of antifungal activity of chitin and chitosan which was increases with increasing concentrations of chitin and chitosan.

*Conflict of interests:* The authors report no conflicts of interest in this work.

*Authors contributions:* M.A. conceived and designed the review. M.A. and W.N. performed the experiments. M.H. wrote the manuscript. M.A. analyzed data and supervise the work. All authors read and approved the manuscript.

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## تاثير الكايتين والكيتوزان في تحسين نمو النبات والنشاط المضاد للفطريات

**ميرفت علي كناوي[1] ، مضر ال حيدر[2] ، وفاء ناصر راضي [2]**
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هدفت الدراسة إلى تحسين إنبات بذور اللوبيا والريحان والخيار من خلال استخدام الكايتين والكيتوزان وتقييم تأثيرهما على بعض الفطريات المسببة للأمراض النباتية. أظهرت النتائج التأثير المشجع للكايتين والكيتوزان على إنبات البذور للمحاصيل الثلاثة. كان لنبات اللوبيا استجابة أكبر للكايتين و الكيتوزان مع وجود فروق معنوية بالمقارنة مع عينات التحكم. وكانت نسب الإنبات 80% و 87% عند معالجتها بتركيز 1 ملجم كايتين أو كيتوزان على التوالي. في حين كانت نسبة إنبات بذور الريحان 91% و 94% عند استخدام 1 ملجم / ملم من الكايتين أو الكيتوزان على التوالي مقارنة بعينة التحكم 76%. من ناحية أخرى، كانت نسبة الانبات لبذور الخيار 96% لكل من 1 ملجم / مل من الكايتين والكيتوزان مقارنة بـ 76% لعينة التحكم. وكانت نسبة الإنبات تتناقص مع زيادة تركيز الكايتين أو الكيتوزان.

أعلى نسبة تثبيط للفطريات الممرضة كانت 70.58% في فطر *Macrophomina* sp. باستخدام 4 ملجم\ ملم كايتن والكيتوزان واقل نسبة تثبيط بتركيز 1 ملجم \ ملم كايتين كانت 8.23% للفطر *A. niger* بينما في وجود 1 ملجم / ملم من الكيتوزان كانت أقل نسبة تثبيط 11.76% في *Rhizoctonia solanni*.

يمكن الاستنتاج أن نسبة إنبات كل من اللوبيا والريحان والخيار تتناقص مع زيادة تركيز الكايتين أو الكيتوزان. كما أن نسبة تثبيط الفطريات الممرضة زادت بزيادة تركيز الكايتين أو الكيتوزان.